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Digestible and metabolizable energy of crude glycerol for growing pigs[1,2]

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ABSTRACT: The apparent DE and ME values of crude glycerol for growing pigs were determined in 5 experiments using crude glycerol (86.95% glycerol) from a biodiesel production facility, which used soybean oil as the initial feedstock. Dietary treatments were 0, 5, or 10% glycerol addition to basal diets in Exp. 1; 0, 5, 10, or 20% glycerol addition to basal diets in Exp. 2; and 0 and 10% crude glycerol addition to the basal diets in Exp. 3, 4, and 5. Each diet was fed twice daily to pigs in individual metabolism crates. After a 10-d adjustment period, a 5-d balance trial was conducted. During the collection period, feces and urine were collected separately after each meal and stored at 0°C until analyses. The GE of each dietary treatment and samples of urine and feces from each pig were determined by isoperibol bomb calorimetry. Digestible energy of the diet was calculated by subtracting fecal energy from the GE in the feed, whereas ME was calculated by subtracting the urinary energy from DE. The DE and ME values of crude glycerol were estimated as the slope of the linear relationship between either DE or ME intake from the experimental diet and feed intake. Among all experiments, the crude glycerol (86.95% glycerol) examined in this study was shown to have a DE of 3,344 ± 8 kcal/kg and an ME of 3,207 ± 10 kcal/kg, thereby providing a highly available energy source for growing pigs.

Key words: biofuel, crude glycerol, metabolizable energy, pig

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INTRODUCTION

Crude glycerol is the principal coproduct of biodiesel production (Ma and Hanna, 1999; Van Gerpen, 2005; Thompson and He, 2006), with 79 g of crude glycerol generated for every 1 L of biodiesel produced (Thompson and He, 2006). With current biodiesel production capacity, approximately 4.16×10^8 kg of crude glycerol could be generated annually (NBB, 2007). Multiple reviews on the metabolic effects of glycerol have been published (Lin, 1977; Tao et al., 1983; Brisson et al., 2001). Glycerol is absorbed by the gastrointestinal tract of nonruminants (Tao et al., 1983) and is utilized as an energy source (Cryer and Bartley, 1973). Glycerol is gluconeogenic, with gluconeogenesis being limited by the availability of glycerol (Cryer and Bartley, 1973; Tao et al., 1983; Baba et al., 1995).

Studies examining the effects of supplementing crude glycerol to diets fed to swine (Mourot et al., 1994; Kijora et al., 1995, 1997; Kijora and Kupsch, 2006) and broilers (Simon et al., 1996; Cerrate et al., 2006) have shown little to no effect on animal performance. Research determining the energy value of crude glycerol is limited. Recently, Bartelt and Schneider (2002) reported a decrease in the ME of glycerol as the level of dietary glycerol was increased in swine and poultry diets. In contrast, Dozier et al. (2008) in broilers and Lammers et al. (2008) in laying hens did not observe this effect.

The objectives of the current study were to determine the apparent DE and ME of crude glycerol at various levels of supplementation and to determine if the apparent energy values differed between starter and finisher pigs.

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[2]Appreciation is given to AG Processing Inc. (Sergeant Bluff, IA) for providing the crude glycerol used in this research.

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Table 1. Characterization of the crude glycerol utilized in the 5 experiments

Item	Value	Analytical method
Total glycerol,[1] %	86.95	ASTM[2] D 6584-00E01
Methanol,[1] %	0.028	Gas chromatography (proprietary method)
pH[1]	5.33	Orion 230A pH meter with 9107 BN probe
Total fatty acid,[1] %	0.29	AOCS[3] G 4.40 modified for glycerin
Moisture,[4] %	9.22	AOAC[5] 984.20
CP,[4] %	0.41	AOAC 990.03
Crude fat,[4] %	0.12	AOAC 920.39 (A)
Ash,[4] %	3.19	AOAC 942.05
Sodium,[4] %	1.26	AOAC 956.01
Chloride,[4] %	1.86	AOAC 9.15.01, 943.01
Potassium,[4] %	<0.005	AOAC 956.01
Color[4]	<1	AOCS Cc 13a-43
GE,[6] kcal/kg	3,625 ± 26	Isoperibol bomb calorimeter

[1]Values reported by AGP Inc., Sergeant Bluff, IA; Lot # GB605-03.
[2]American Society for Testing Materials (2006).
[3]American Oil Chemists' Society (2000).
[4]Analysis by University of Missouri-Columbia Experiment Station Chemical Laboratories, Columbia, MO.
[5]Association of Official Analytical Chemists (1995).
[6]Analysis by USDA-ARS, Ames, IA; Model number 1281, Parr Instrument Co. Inc., Moline, IL.

MATERIALS AND METHODS

General Pig Management

The Iowa State University Animal Care and Use Committee approved all experimental protocols.

All experiments utilized the same batch of crude glycerol (86.95% glycerol). The crude glycerol was characterized through standard techniques (AOAC, 1995; AOCS, 2000; ASTM, 2006) and is detailed in Table 1. Crude glycerol was obtained from biodiesel production using soybean oil as the initial feedstock (AG Processing Inc., Sergeant Bluff, IA). Three experiments (Exp. 1, 3, and 4) examined crude glycerol fed to starter pigs (average initial BW, 10.3 ± 1.4 kg), whereas 2 experiments (Exp. 2 and 5) examined crude glycerol fed to finishing pigs (average initial BW, 104.7 ± 8.0 kg). In each experiment, 24 pigs were randomly assigned to individual metabolism crates equipped with screens and trays that allowed total but separate collection of feces and urine. Dimensions of the individual metabolism crates were 0.53 × 0.71 m for starter pigs and 0.8 × 2.1 m for finishing pigs. Due to the crate design, barrows were used in the starter pig metabolism experiments, whereas gilts were used in the finishing pig metabolism experiments.

Pigs were randomly assigned to dietary treatments after pen assignment. Dietary treatments consisted of a common basal diet, which met or exceeded the NRC requirements (NRC, 1998), and 0, 5, 10, or 20% crude glycerol addition to the basal diet (Exp. 1 and 2) or 0 or 10% crude glycerol addition to the basal diet (Exp. 3, 4, and 5). Basal diet formulations and calculated analyses are summarized in Table 2. A 10-d adjustment period was used to adapt the pigs to the metabolism crate and the dietary treatment.

Pigs were fed 2 equal daily meals. In Exp. 1, 2, 4, and 5, pigs were fed a set amount of the basal diet, with pigs on the glycerol treatments offered an increased feed allotment based on the amount of glycerol addition to the basal diet (Adeola, 2001). In Exp. 3, pigs assigned to 10% crude glycerol received only 5% more feed than control pigs. In all experiments, pigs were fed twice daily, with feed consumption and refusal recorded at the end of the experimental period. Table 3 details the daily feed allowance and components for dietary treatments fed for each of the 5 experiments. Following the adjustment period, urine was collected for 5 d into stainless steel buckets containing 30 mL of 6 *N* HCl placed below the collection drain of each crate. Urine was collected twice daily, diluted with water to a constant volume, and thoroughly mixed, with a representative aliquot collected and stored at 0°C until subsequent analysis.

In Exp. 1 and 2, Fe_2O_3 (0.25% wt:wt) was thoroughly mixed with the initial feed allocation and fed on the evening of d 10. The appearance of the marker in the feces signaled the beginning of the fecal collection period. Feces were collected twice daily and stored at 0°C. A second pulse of Fe_2O_3 was thoroughly mixed and fed with the 10th meal (5-d collection period). Upon appearance of the second pulse of marker in the feces, collection was terminated. Because pigs seemed to have aversion to the feed containing the marker in Exp. 1 and 2, no marker was utilized in Exp. 3, 4, and 5. Rather, total fecal collection was performed for 120 h (5 d) beginning on the morning of d 11 and ending on the morning of d 16.

Chemical Analyses

Feed samples were ground through a 1-mm screen before energy determination. Fecal samples were thawed, dried at 70°C for 48 h, and weighed to determine the DM content. Fecal samples were ground through a 1-mm screen in preparation for energy deter-

Table 2. Ingredient and calculated content of basal diets fed to starter and finisher pigs, as-fed basis

Item	Starter[1]	Finisher[2]
Ingredient, %		
Corn	44.75	79.20
Soybean meal, 47.5% CP	38.92	18.30
Whey (dried)	12.50	0.00
DL-Met	0.03	0.00
L-Thr	0.02	0.00
Dicalcium phosphate	1.84	0.90
Limestone	1.00	0.85
Sodium chloride	0.25	0.33
Trace mineral mix	0.15[3]	0.09[4]
Choline chloride, 60%	0.03	0.00
Vitamin mix	0.37[5]	0.20[6]
Mold inhibitor	0.10	0.10
Total	100.00	100.00
Calculated content		
ME, Mcal/kg	3.326	3.327
Lys, %	1.40	0.76
TSAA, %	0.79	0.54
Thr, %	0.96	0.57
Trp, %	0.30	0.17
Ca, %	1.02	0.60
Available P, %	0.51	0.23
Na, %	0.23	0.15
Cl, %	0.37	0.25

[1]Mean initial BW, 10.3 ± 1.4 kg.
[2]Mean initial BW, 104.7 ± 8.0 kg.
[3]Provided the following per kilogram of diet: Cu, 26.3 mg as Cu oxide; Fe, 263 mg as Fe sulfate; I, 3.0 mg as Ca iodate; Mn, 90 mg as Mn oxide; and Zn, 225 mg as Zn oxide.
[4]Provided the following per kilogram of diet: Cu, 15.8 mg as Cu oxide; Fe, 158 mg as Fe sulfate; I, 1.8 mg as Ca iodate; Mn, 54 mg as Mn oxide; and Zn, 135 mg as Zn oxide.
[5]Provided the following per kilogram of diet: vitamin A, 8,157 IU; vitamin D_3, 2,039 IU; vitamin E, 41 IU; vitamin B_{12}, 0.04 mg; riboflavin, 12.2 mg; niacin, 61.2 mg; and D-pantothenic acid, 32.6 mg.
[6]Provided the following per kilogram of diet: vitamin A, 4,409 IU; vitamin D_3, 1,102 IU; vitamin E, 22 IU; vitamin B_{12}, 0.02 mg; riboflavin, 6.6 mg; niacin, 33.1 mg; and D-pantothenic acid, 17.6 mg.

mination. For urine energy determination, 2 mL of urine was added to 0.5 g of dried cellulose and subsequently dried at 50°C for 24 h before energy determination. The GE of feed, feces, and urine plus cellulose was determined using an isoperibol bomb calorimeter (model number 1281, Parr Instrument Co., Moline, IL), with benzoic acid used as a standard. Duplicate analyses were performed on all diets and fecal samples from each pig, whereas triplicate analyses were performed on diluted urine plus cellulose from each pig. Urinary energy was determined by subtracting the energy contained in cellulose from the combined urine plus cellulose energy.

Calculations and Statistical Analysis

Observations from 108 pigs of the 120 pigs assigned to dietary treatments among all experiments were used for analysis. Observations from 9 pigs were not possible to quantify due to diarrhea, constipation, or feed refusal. Observations from 3 pigs exceeded their treatment group mean by more than 2 SD and were considered outliers. The authors do not have an explanation why all but 1 pig excluded from analysis received experimental diets containing crude glycerol.

Gross energy consumed was calculated by multiplying the GE value of the diet fed by feed intake over the 5-d collection period. Apparent DE values were calculated by subtracting fecal energy from intake energy. Apparent ME values were calculated by subtracting urinary energy from apparent DE. The apparent DE and ME values of crude glycerol fed to pigs were estimated as the slope of the linear relationship between the apparent DE or ME intake from the experimental diet, the dependent variable, and feed intake, the independent variable (Adeola, 2001; JMP 6.0, SAS Inst. Inc., Cary, NC). A regression model was used to test for the effect of feed intake, experiments, fecal collection

Table 3. Number of pigs, daily feed allowance, and components fed for the 5 experiments[1]

Exp.	Glycerol addition, %	No. of pigs	Daily intake		GE, kcal/kg of diet
			Basal diet, g	Glycerol, g	
1 (initial BW = 11.0 ± 0.5 kg)[2]	0	6	376	0	3,680
	5	6	376	19	3,670
	10	6	376	38	3,707
	20	6	376	75	3,681
2 (initial BW = 109.6 ± 5.5 kg)[2]	0	6	2,292	0	3,652
	5	6	2,292	115	3,666
	10	6	2,292	229	3,664
	20	5	2,292	458	3,690
3 (initial BW = 8.4 ± 0.9 kg)[3]	0	12	316	0	3,746
	10	7	300	30	3,806
4 (initial BW = 11.3 ± 0.7 kg)[3]	0	11	400	0	3,778
	10	9	400	40	3,780
5 (initial BW = 99.9 ± 7.4 kg)[3]	0	12	2,000	0	3,783
	10	10	2,000	200	3,768

[1]Pigs were fed 2 equal meals daily in each experiment.
[2]Fecal collection by the marker method.
[3]Fecal collection by the 120-h method.

Table 4. Apparent energy values for the 5 experiments[1]

Item	Glycerol addition, %			
	0	5	10	20
Exp. 1 (initial BW, 11.0 ± 0.5 kg)				
GE intake, kcal/d	1,384 ± 13	1,450 ± 16	1,535 ± 1	1,660 ± 5
Fecal energy, kcal/d	147 ± 19	138 ± 14	146 ± 21	168 ± 19
DE, kcal/d	1,237 ± 19	1,311 ± 14	1,389 ± 21	1,491 ± 19
Urinary energy, kcal/d	47 ± 16	56 ± 19	68 ± 25	108 ± 25
ME, kcal/d	1,190 ± 30	1,255 ± 25	1,321 ± 36	1,384 ± 29
Exp. 2 (initial BW, 109.6 ± 5.5 kg)				
GE intake, kcal/d	8,370 ± 46	8,824 ± 8	9,237 ± 64	10,148 ± 89
Fecal energy, kcal/d	798 ± 108	811 ± 48	885 ± 83	828 ± 50
DE, kcal/d	7,573 ± 108	8,013 ± 48	8,352 ± 83	9,320 ± 50
Urinary energy, kcal/d	298 ± 28	282 ± 24	350 ± 40	600 ± 44
ME, kcal/d	7,277 ± 124	7,731 ± 53	8,002 ± 81	8,720 ± 83
Exp. 3 (initial BW, 8.4 ± 0.9 kg)				
GE intake, kcal/d	1,180 ± 1		1,256 ± 1	
Fecal energy, kcal/d	121 ± 14		115 ± 9	
DE, kcal/d	1,059 ± 14		1,141 ± 9	
Urinary energy, kcal/d	48 ± 8		61 ± 19	
ME, kcal/d	1,011 ± 18		1,080 ± 23	
Exp. 4 (initial BW, 11.4 ± 0.7 kg)				
GE intake, kcal/d	1,511 ± 2		1,663 ± 10	
Fecal energy, kcal/d	160 ± 21		150 ± 16	
DE, kcal/d	1,352 ± 21		1,514 ± 16	
Urinary energy, kcal/d	53 ± 7		73 ± 12	
ME, kcal/d	1,299 ± 23		1,441 ± 18	
Exp. 5 (initial BW, 99.9 ± 7.4 kg)				
GE intake, kcal/d	7,566 ± 27		8,290 ± 33	
Fecal energy, kcal/d	858 ± 136		836 ± 86	
DE, kcal/d	6,708 ± 136		7,451 ± 86	
Urinary energy, kcal/d	198 ± 38		264 ± 31	
ME, kcal/d	6,510 ± 158		7,187 ± 90	

[1]Calculated energy values presented as means ± SEM.

method, type of pig, and the type of pig × feed intake interaction on apparent DE and ME.

RESULTS AND DISCUSSION

Production of biofuels is increasing due to rising energy prices, uncertain access to petroleum supplies, and recognition of the environmental impacts of fossil fuel use (Ma and Hanna, 1999; Hill et al., 2006; Kurki et al., 2006). Consequently, increased production of coproducts from biofuels industries will necessitate livestock producers to be flexible in feedstuff choice. Crude glycerol, a readily available energy source, may play an important role in meeting the energy needs of pigs as biodiesel production expands.

The ME of the basal diets used in the starter experiments were 3,165, 3,199, and 3,248 kcal/kg for Exp. 1, 3, and 4, respectively (Tables 3 and 4). The ME of the basal diets used in the finisher experiments were 3,174 and 3,255 kcal/kg for Exp. 2 and 5, respectively (Tables 3 and 4). These values are within 5% of those calculated for the starter and finisher basal diets (Table 2) and reflect good collection and analytical techniques in all experiments. The GE of crude glycerol evaluated in these experiments was determined to be 3,625 ± 26 kcal/kg. This is similar to expectations relative to pure glycerol (in-house GE analysis of 4,305 kcal/kg), given that our sample of crude glycerol evaluated contained 86.95% glycerol with low concentrations of methanol (0.028%) and free fatty acids (0.29%). Based on our

Table 5. Apparent energy values of crude glycerol fed to pigs, as-fed basis[1]

Exp.	Pigs	Initial BW, kg	DE, kcal/kg	SEM	ME, kcal/kg	SEM
1	18	11.0 ± 0.6	4,401	282	3,463	480
2	23	109.6 ± 5.5	3,772	108	3,088	118
3	19	8.4 ± 0.9	3,634	218	3,177	251
4	20	11.3 ± 0.7	4,040	222	3,544	237
5	22	99.9 ± 7.4	3,553	172	3,352	192

[1]All experiments represent data from 5-d energy balance experiments following a 10-d adaptation period.

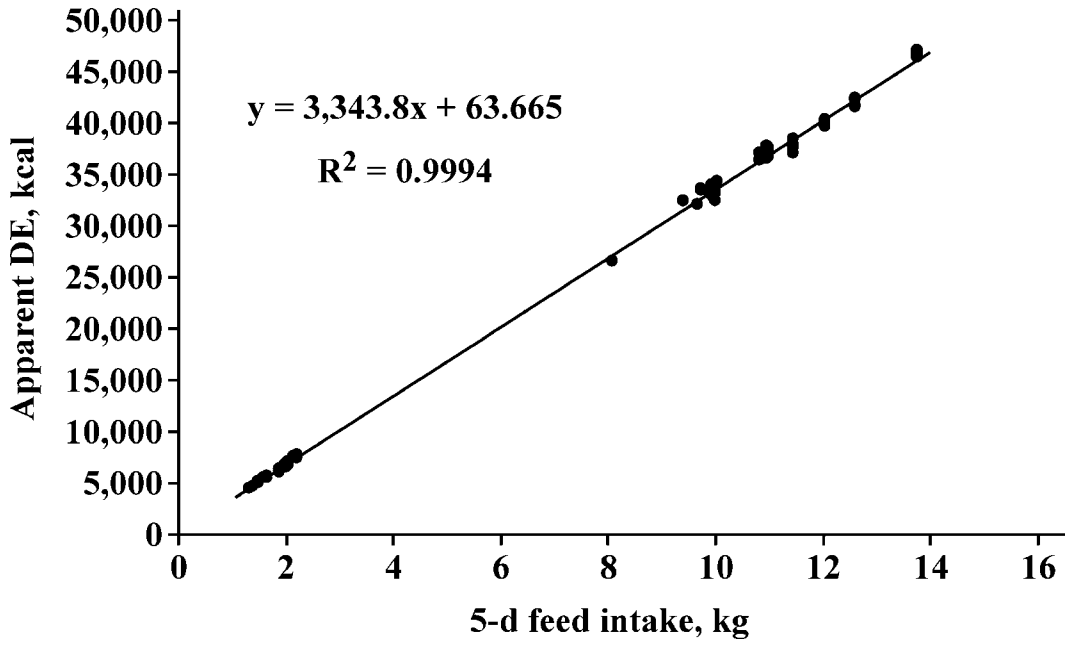

Figure 1. Apparent DE of crude glycerol fed to pigs. Data represent the combined regression from Exp. 1 through 5 of DE intake over feed consumption for a 5-d period for 102 pigs fed 0, 5, 10, or 20% crude glycerol, with the slope of the regression line indicating that the DE for crude glycerol equals 3,344 kcal/kg.

data in broilers (Dozier et al., 2008) and laying hens (Lammers et al., 2008), we did not expect the level of crude glycerol to affect ME determination. However, when data from Exp. 1 were analyzed separately, the ME of crude glycerol declined with increasing levels of supplementation, with estimated ME values of 3,601, 3,239, and 2,579 kcal/kg crude glycerol for 5, 10, and 20% inclusion levels, respectively (quadratic, $P = 0.05$). Bartelt and Schneider (2002) also showed a decrease in the ME of glycerol (99.9% glycerol) with increasing levels of glycerol fed to 34-kg barrows, with ME/kg being 4,177, 3,436, and 2,524 kcal/kg for 5, 10, and 15% inclusion levels, respectively. In Exp. 1, the decrease in ME of glycerol seems to be due to pigs fed the 20% crude glycerol. Removing the 20% inclusion level data from Exp. 1 resulted in no such difference in ME estimation with the remaining levels of crude glycerol (0, 5, and 10%) resulting in a ME value of 3,463 kcal/kg (linear, $P = 0.001$). In contrast, there was no effect of crude glycerol inclusion level on the ME estimate when determined with finishing pigs in Exp. 2.

Apparent energy values for all 5 experiments are detailed in Table 4. Among all treatments, digestibility ranged between 89 and 92%, whereas ME values were between 86 and 88% of GE intake. The only exception was observed for starter pigs fed 20% crude glycerol (Exp. 1). The digestibility was 90% in those 6 pigs; however, the ME value was 83% of the GE intake. This further highlights a potential decline in the ability of 11-kg pigs to metabolize more than 10% of crude glycerol. We do not have an explanation for this effect because enzyme kinetics involved in glycerol metabolism have not been studied in the pig, and this experiment was not designed to evaluate tissue utilization of glycerol in the pig. With 6 starter pigs fed 20% crude glycerol, it is difficult to draw conclusions about the small pig's ability to utilize crude glycerol, although the question should be examined further. Given the fact that pigs fed the 20% crude glycerol in Exp. 1 had reduced utilization of crude glycerol, as determined by a decreased ME estimate, we chose to exclude those pigs from subsequent analysis.

Markers such as Fe_2O_3 have long been used in nutritional studies (Kotb and Luckey, 1972). In Exp. 1 (starter) and 2 (finisher), Fe_2O_3 seemed to affect palatability of the diet through visual evaluation of feed acceptance at the initiation of the collection period. This is supported by Jagger et al. (1992) who reported that 57-kg pigs had some initial reluctance to consume feed when the level of marker was increased from 0.1 to 0.5% TiO_2. We chose not to use a marker in Exp. 3, 4, and 5 because acceptance of feed is critical in short-term metabolic studies.

Table 5 presents the apparent DE and ME values as determined by linear regression (Adeola, 2001) for Exp. 1 to 5. Apparent DE and ME were not affected by experiment (Exp. 1 to 5), use of marker to determine fecal collection time points (Exp. 1 and 2 vs. Exp. 3, 4, and 5), type of pig (starter, Exp. 1, 3, and 4 vs. finisher, Exp. 2 and 5), or by type of pig × feed intake interaction. As expected, feed intake affected both apparent DE and ME intake ($P \leq 0.001$).

In the current experiments, the ratio of DE:GE for the crude glycerol examined equaled 92%, indicating that crude glycerol was well digested by pigs. In comparison to corn and soybean oil, 2 common feedstuffs used

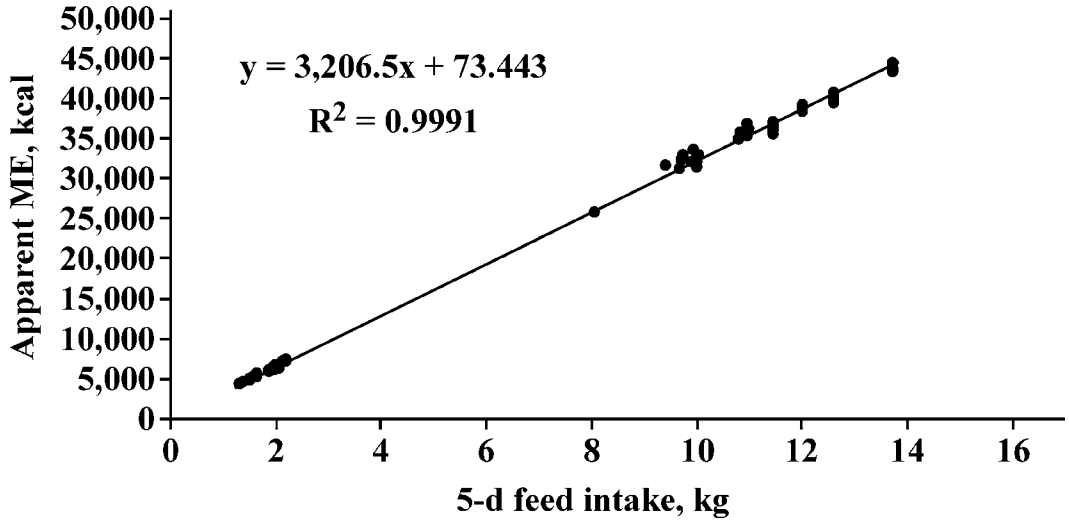

Figure 2. Apparent ME of crude glycerol fed to pigs. Data represent the combined regression from Exp. 1 through 5 of ME intake over feed consumption for a 5-d period for 102 pigs fed 0, 5, 10, or 20% crude glycerol, with the slope of the regression line indicating that the ME for crude glycerol equals 3,207 kcal/kg.

to provide energy in pig diets, the ratio of ME:DE for the crude glycerol examined was 96%, which is identical to the ME:DE ratio for soybean oil and is comparable with the ratio of ME:DE for corn, which is 97% (NRC, 1998). These relationships support the assertion that the crude glycerol used in these experiments was well utilized by the pig as a source of energy. This is in agreement with Bartelt and Schneider (2002) who reported that >97% of the glycerol is digested prior to the cecum.

The results of combined regressions indicated that the DE value of the examined crude glycerol (86.95%) was 3,344 ± 8 kcal/kg (Figure 1), and the ME was 3,207 ± 10 kcal/kg (Figure 2). Recent work with the same crude glycerol estimated an apparent ME (corrected for nitrogen) to be 3,805 kcal/kg for laying hens (Lammers et al., 2008) and 3,434 for broilers (Dozier et al., 2008), which are not different from the reported GE for this sample of crude glycerin (3,625 ± 26 kcal/kg). Tao et al. (1983) indicated that the oxidation of glycerol to carbon dioxide releases 4,320 kcal/kg. Rosebrough et al. (1980) assumed a ME value of 4,200 kcal/kg for dietary glycerol in turkeys, whereas Cerrate et al. (2006) estimated a ME value of 3,528 kcal/kg in broilers. Until now, no work has reported an actual determination of ME of crude glycerol in swine. When placed on an equivalent glycerol basis, our ME determination would be marginally greater than the 3,436 kcal of ME/kg determined for pure glycerol (Bartelt and Schneider, 2002).

With an ME of 3,207 ± 10 kcal/kg, crude glycerol can be used as an excellent source of energy for growing pigs. Concentrations of other compounds in crude glycerol (i.e., methanol, sodium- or potassium chloride, and free fatty acids), however, must be monitored to prevent excessive amounts in pig diets and for potential impacts on ME determination of this feedstuff.

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